

## Preprocess of the milk samples

### Demonstration:

Preprocess of milk samples:

Mixing 1 mL milk sample with 4 mL PBS buffer (pH=7.2), 100  $\mu$ L penicillin (3 times diluted range from 3000ppm to 3ppm, plus one sample adding no penicillin), 5 mL n-hexane

Vibrating for 10 mins

Centrifuging at 8000rpm and 15 °C for 10 mins

Collecting the under layer liquid

1. Adding eight preprocessed milk samples to each well in column 2
2. Adding 100  $\mu$ L supernatant (containing PBP-GFP) is added to each well in column 2.  
Incubate in 37°C for 0.5h

similar to the third competitive binding experiment, we have proved that as the concentration of penicillin in milk samples increases, the Florence intensity in the supernatant increases. Though less strongly related the data is, the experiment indicates that our method is still applicable when milk presents in the system.